CARBON FIBER OBSCURANT: ENHANCING WARFIGHTER EFFECTIVENESS WHILE MEETING ENVIRONMENTAL REGULATIONS

Carlton T. Phillips, Ronald T. Checkai*, Roman G. Kuperman, Michael Simini, and Nancy A. Chester

U.S. Army Edgewood Chemical Biological Center (ECBC)
Aberdeen Proving Ground, MD 21010-5424 USA

ABSTRACT

Although the battlefield scenario has changed over the last decade, the need still exists for an obscurant capable of countering enemy weapon strikes. A millimeter-wave (MMW) material obscuring in the 9-96 GHz range of the electromagnetic spectrum has become critical for the modern Warfighter in order to minimize radar detection by hostile forces, thus saving lives and equipment. To meet this end, the Warfighter must be highly trained in all tasks across the spectrum of military operations. These Warfighters will need demanding, highly realistic training to obtain this capability. This increased training will lead to increased releases into the environment of obscurants at training sites. An uncoated carbon fiber obscurant (CFO) has been developed for a new module of the M56E1 smoke generation system. In order to field this new capability in 2008, we investigated the potential for environmental impacts of CFO in soil thereby supporting thorough environmental analysis and documentation with the goals of: 1) minimal restrictions on troop testing and training, and 2) sustainable range use. Ecotoxicological investigation of CFO in soil included invertebrate toxicity tests (potworm, earthworm, Collembola), plus a microcosm bioassay with the indigenous microarthropod and nematode communities. Uncoated CFO had no significant adverse effect on adult survival or reproduction of test species in single-species toxicity tests at concentrations of CFO in soil up to 10,000 mg kg^{-1}. In addition, soil microinvertebrate community groups in the microcosm bioassay were unaffected up to the highest concentration tested (1,272 mg kg^{-1}). These test results for CFO comply with environmental regulations, and support testing and training requirements and Army mission.

1. INTRODUCTION

Obscurants can be used to counter enemy weapon strikes on the battlefield, and are also important to Special Operations. An uncoated carbon fiber obscurant (CFO) MMW module has been developed for the M56E1 smoke generation system via the MMW Pre-Planned Product Improvement (P^3I) Program. The CFO MMW module provides the advanced capability for delivering large area obscurant screens in the millimeter wave-range while maintaining the capabilities already existing in the infrared (IR; graphite flakes) and visual (fog oil) ranges. The Army spends approximately $1.5B annually on environmental quality management; 75% of Army environmental liability and costs at installations comes from operation and sustainment of fielded systems. Because the newly modified M56E1 is scheduled for fielding in 2008, an Environmental Research Program (ERP) was instituted to meet the requirements of Department of Defense Instruction (5000.2 Defense Acquisition) and the National Environmental Policy Act (NEPA). The new Army Strategy for the Environment: Sustain the Mission – Secure the Future (U.S. Army ASAIE, 2004) establishes a long-range vision that enables the Army to meet its mission today and into the future. Sustainability is the foundation for this strategy, and a paradigm that focuses our thinking on addressing both present and future needs while strengthening community partnerships that improve our ability to organize, equip, train, and deploy our soldiers as part of the joint force. Sustainability connects our activities today to those of tomorrow with sound business and environmental practices. Objective Force Soldiers must receive highly realistic training across the spectrum of military operations. Realistic training leads to increased releases of materials such as CFO into the environment at training and testing sites. We assessed the potential for ecological impact from the release of CFO into the environment by generating species-specific and community-level ecotoxicological data for soil biological receptors.

2. MATERIALS AND METHODS

2.1 Carbon Fiber Obscurant

CFO was collected from an M56E1 generator. A nylon hose was attached to the outlet of the generator to capture disseminated CFO. The generator was turned on for several minutes until the hose was 75% to 100% full. This procedure was repeated several times. The disseminated CFO was stored in a dry, dark, temperature-controlled room until used in bioassays.

2.2 Soil Amendment Procedures

Treatment concentrations of CFO for toxicity testing were prepared by adding appropriate amounts, from the

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 NOV 2006	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Carbon Fiber Obscurant: Enhancing Warfighter Effectiveness While Meeting Environmental Regulations	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Army Edgewood Chemical Biological Center (ECBC) Aberdeen Proving Ground, MD 21010-5424 USA**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM002075., The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**7**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

whole amount of disseminated CFO material, to 200 g (soil dry weight) portions of the natural soil Sassafras Sandy Loam [Fine-loamy, siliceous, mesic Typic Hapludult] (USDA/ARS, 1999; SSL) in individual replicate test containers to achieve nominal target concentrations of 0 (negative control), 100, 1000, and 10000 mg kg^{-1} (dry soil mass, DM). Test concentrations of CFO in soil were determined based on best estimates from a dispersion modeling study (STC, 2004, 2005). Estimated mean deposition for a 60-minute dissemination at 3,629 g CFO min^{-1}, ranges from 1,186 g m^{-2} at 10 m from the source to 3 g m^{-2} at 200 m, assuming 6 generators spaced 50 m apart, using 24 um average aerodynamic equivalent diameter with an exit velocity of 0.01 m s^{-1} and plume depletion, as inputs to the Industrial Source Code Short-Term (ISCST3) model. These deposition rates convert to a mean soil concentration of 6,248 mg kg^{-1} assuming an effective depth of incorporation into SSL soil of 5 cm.

Soil was hand-mixed with a spatula to homogeneously distribute CFO material into the test matrix. After mixing, soil was hydrated to 100%, 88%, and 95% of the Water Holding Capacity (WHC, 18% water on the basis of the dry SSL soil mass), for toxicity testing with potworms, springtails, and earthworms, respectively, and allowed to equilibrate 24 h before adding the organisms to the soil.

2.3 Sassafras Sandy Loam Soil

SSL soil was used in the ecotoxicological studies of CFO. The SSL soil was collected from an open grassland field on the property of the U.S. Army Aberdeen Proving Ground (APG, MD). Vegetation and the organic matter horizon were removed to just below the root zone, and the top 15 cm of the A horizon were then collected. The soil was sieved through a 5-mm^2 mesh screen, air-dried for at least 72 h and mixed periodically to ensure uniform drying, then passed through a 2-mm sieve, and stored at room temperature before use in testing. Soil was analyzed for physical and chemical characteristics by The Penn State University Agricultural Analytical Lab. Results of these analyses are presented in Table 1.

Table 1. Physical and Chemical Characteristics of Sassafras Sandy Loam Soil Analyzed by the Penn State University Agricultural Analytical Laboratory.

Soil Parameter	Sassafras Sandy Loam
Sand %	74
Silt %	9
Clay %	16
Texture	Sandy loam
CEC cmol kg^{-1}	7.0
Organic matter %	0.7
pH	5.3

2.4 Standardized Toxicity Tests

Standardized single-species toxicity tests included Enchytraeid Reproduction Test (ISO, 2005), Earthworm Reproduction Test (ISO, 1998), and Collembola Reproduction Test (ISO, 1999). Test species selected for our studies were representative surrogates of ecologically relevant species that normally inhabit a wide range of soils and geographical areas, and the measurement endpoints for the bioassays selected included at least one endpoint based on reproduction.

2.4.1 Enchytraeid Reproduction Test

The Enchytraeid Reproduction Test (ERT) was used to assess the effects of CFO material on the reproduction of potworm *Enchytraeus crypticus*. The test is an adaptation of an ISO bioassay, Soil Quality-Effects of Pollutants on Enchytraeidae (*Enchytraeus* sp.)- Determination of effects on reproduction and survival (ISO, 2005), which was modified for use with natural soils. The ERT is a Chronic/Life-Cycle Assay. The ISO Guideline for this assay was originally developed for use with OECD artificial soil (USEPA Standard Artificial Soil; USEPA, 1989) however our research showed that this test might also be conducted using natural soils (Kuperman, et al., 1999). The ISO ERT was designed for use with the enchytraeid worm species *E. albidus.* Results of our previous studies using *E. albidus* showed that this species requires soils containing high organic matter content with a soil pH 6 (±0.5) for optimal test conditions; this species performed poorly in natural soils with physical and chemical characteristics that support a higher level of chemical bioavailability (Kuperman et al., 1999; 2003; 2004; 2005; 2006). We selected the species of Enchytraeidae, *E. crypticus*, which is listed in the ISO protocol as an acceptable alternative to *E. albidus* and is suitable for toxicity testing in SSL soil.

2.4.1.1 Test Performance – ERT

We hypothesized that CFO material tested in these studies would not be highly toxic to soil invertebrates. This hypothesis was based on the fact that CFO is similar to recalcitrant (*i.e.* relatively non-reactive and resistant to microbial degradation) carbon compounds that occur naturally as a result of organic matter decomposition in soil ecosystems, associated with overall improvement and maintenance of soil quality and sustainable functioning of the soil biota community. Furthermore, a previous study with earthworms showed no mortality and only a slight reduction in growth with CFO concentrations greater than 10,000 mg kg^{-1} in soil (Cataldo et al., 1992). Based on this information, we designed a composite toxicity test with variable replication of individual treatments. This design combined a range-finding test component of the ERT and a definitive Limit Test component. In the range-finding test component, a limited number of treatment

concentrations and replicates were used to determine the concentration range for the definitive study, if required. The Limit Test component was based on using increased replication in the control and in the highest treatment concentration (10,000 mg kg^{-1}). The Limit Test is a variant of a definitive test that is performed when statistical analysis of the range-finding test data shows no significant effect at all treatment levels. The composite range-finding/Limit toxicity test design used in this study provided a range of test concentrations of CFO that may naturally occur during field testing, plus sufficient replication for the Limit Test. This bioassay included the CFO negative control (no CFO added), plus the positive control (added reference toxicant) and its separate negative control (no added reference toxicant). Positive control exposures were prepared from a solution of beryllium sulfate ($BeSO_4 \cdot 4H_2O$, CAS 7787-56-6, purity 99.99%, Alfa Aesar, Ward Hill, MA, USA) in ASTM Type I water, to produce 45 mg kg^{-1} beryllium (Be) nominal concentrations in soil; these positive control bioassays were conducted periodically throughout each year of testing in order to monitor the performance of laboratory culture of *E. crypticus*. Treatment replication in the composite toxicity test design was as follows: negative control for CFO – 8 replicates; 100 mg kg^{-1} – 2 replicates; 1,000 mg kg^{-1} – 2 replicates; 10,000 mg kg^{-1} – 8 replicates; Be control – 4 replicates; negative control for Be – 4 replicates.

Exposures were conducted in glass test jars (42 mm internal diameter (ID); 45 mm deep), each containing twenty grams (DM) of prepared soil. Ground oats (0.05 g) were mixed with soil in each test container to provide food for potworms. Adult potworms with eggs in the clitellum region were collected from culture. Ten worms selected for uniformity (approximately 1 cm in length) were placed on top of prepared soil in each test container. Plastic wrap was stretched over the top of each container and secured with a rubber band. Three pinholes were made in the plastic wrap to facilitate air exchange. All containers were placed in an environment-controlled incubator at 22±1°C and 16:8 h light:dark photoperiod. The containers were weighed at the start of the test. Each container was then weighed once each week and water loss replenished with ASTM type I water to the respective original weights. Ground oats (0.05 g) were added to each test container at that time.

After two weeks, soil in each test container was carefully examined and adult potworms were removed and counted. The remaining test substrate, including any cocoons laid during the first two weeks of the test, was incubated for an additional two weeks. After four weeks from the start of the test, soil in the test containers was fixed with 70% ethanol, and nine drops of Rose Bengal biological stain (1% solution in ethanol) were added. Staining continued for a minimum of 24 h. The content of each test container was wet-sieved using a No. 100 mesh (150 μm) sieve. The retained contents were transferred to a counting tray where potworms were counted. Measurement endpoints were the number of surviving adults after 14 days, and number of juveniles produced after 28 days.

2.4.1.2 Validity of the Test – ERT

Validity criteria were included in the test as part of the Quality Control procedures. They included the following performance parameters for the negative controls:

1) Adult mortality does not exceed 20% after 14 days
2) Average number of juveniles is ≥2x initial number of adults per test container at the end of the test
3) Coefficient of variation for the mean number of juveniles is ≤50% at the end of the test

Test results complied with the validity criteria, defined in the ISO test guideline. Mean adult survival in CFO, and Be negative controls, were 100%. The mean numbers of juveniles in the CFO treatment, and the Be negative control were 1801 and 1655, respectively, and coefficients of variation were 7.3 and 14.9%, respectively. Juvenile production in Be control was reduced by 60% from Be negative control and was within ±2x standard error of the baseline established for the laboratory culture of *E. crypticus*.

2.4.1.3 Data Analysis – ERT

Analysis of Variance (ANOVA) was used to test for significant effect of exposure on adult survival. Mean separations were done using Tukey HSD Multiple Comparisons tests with significance level of $p \leq 0.05$. These statistical analyses were done using SYSTAT® 7(SPSS, Inc., Chicago, IL, USA). Student's *t*-Test (two-tailed) with significance level of $p \leq 0.05$ was used to analyze the juvenile production data in the Limit Test. These statistical analyses were done using Microsoft EXCEL software (Microsoft Corporation, 2003).

2.4.2 Earthworm Reproduction Test

The current standard Earthworm Reproduction Test is Soil Quality – Effects of pollutants on earthworms (*Eisenia fetida*) – Part 2: Determination of effects on reproduction (ISO, 1998). The guidelines for this assay were originally developed for use with OECD artificial soil (USEPA Standard Artificial Soil; USEPA, 1989); however our previous studies showed that this test can also be conducted using natural soils. We adapted the standardized earthworm bioassay, using natural SSL soil in these CFO toxicity studies. A 21-day reproduction test was performed in freshly amended soil, and we assessed both adult survival, and cocoon production as the reproduction endpoint.

2.4.2.1 Test Performance – Earthworm

The goal of this experiment was to determine the potential toxicity of CFO to the earthworm *E. fetida* in SSL soil using a range of concentrations that may be encountered during training and testing exercises.

Earthworms were extracted from earthworm culture beds and depurated on moistened (ASTM Type I water) filter paper for 24 h. CFO fibers were weighed and separated by hand, then placed in alternate layers with soil and subsequently mixed by hand with a spatula within each glass test jar to attain the desired concentrations. Water (34.2 g) was then added to each jar and allowed to equilibrate for approx. 24 h. The moist soil and fiber in each jar were then mixed again by hand with a spatula. Earthworms were removed from filter paper and rinsed three times with ASTM Type I water. Five earthworms were placed in each jar and the jar was sealed with plastic wrap and secured with an open-ringed screw cap. Three pinholes were made in the plastic wrap to allow air exchange. Each concentration was replicated 4x, including negative controls (no added CFO). Positive controls (boric acid toxicant added) were also tested. Positive control exposures were prepared by adding appropriate amounts of a solution of boric acid (H_3BO_3, CAS 10043-35-3, purity 99.99%, Alfa Aesar, Ward Hill, MA, USA) in ASTM Type I water to SSL soil. Positive controls were included in order to monitor the performance of laboratory culture of *E. fetida* and verify consistent sensitivity and response of the test organisms. Jars were then placed in an incubator at 22±1°C, 16:8 light:dark photoperiod, and >70% relative humidity (RH). Earthworms were harvested after three weeks and the number of adult survivors and cocoons were recorded.

2.4.2.2 Validity of the Test – Earthworm

The validity criteria were included in the test as part of the Quality Control procedures. They included the following performance parameters for the negative controls:

1) Mean mortality did not exceed 10% at the end of the range-finding tests
2) Coefficient of variation for the mean number of cocoons was ≤30% at the end of the test.

Test results complied with the validity criteria, defined in the ISO test guideline. The negative control results met the validity criteria given above. Results for positive controls yielded boric acid EC_{50} values for the reproduction endpoints of cocoon production (178 mg kg^{-1} avg.) and juvenile production (74 mg kg^{-1} avg.), and both EC_{50} values were within their respective 95% confidence intervals (CI) as is required for compliance with ISO test guidelines.

2.4.2.3 Data Analysis – Earthworm

Data were analyzed using Analysis of Variance (ANOVA) and Fisher's Least Significant Difference (LSD) pairwise means comparison tests. A significance level of $p<0.05$ was used to determine differences between treatment means. Statistical analyses were performed using SYSTAT® 10.2 (SYSTAT Software, Inc., Richmond, CA, USA).

2.4.3 Collembola Reproduction Test

The Folsomia Reproduction Test was selected to assess the effects of CFO on the reproduction of the Collembolan *Folsomia candida*. The test is an adaptation of an internationally standardized bioassay of the International Standardization Organization (ISO) Soil Quality – Inhibition of Reproduction of Collembola (*Folsomia candida*) by Soil Pollutants (ISO, 1999). The measurement endpoints for the test are adult survival, and production of juveniles. The ISO Guideline for this assay was originally developed for use with OECD artificial soil (USEPA Standard Artificial Soil; USEPA, 1989). Our research has shown that this bioassay can be conducted using natural soils (Phillips et al., 2002).

2.4.3.1 Test Performance – Collembola

The experimental design used for the Folsomia Reproduction test was the Limit Test, which consisted of 10 replicates each of the negative control, and the greatest concentration tested (10,000 mg kg^{-1} CFO), plus 5 replicates of positive control consisting of beryllium sulfate ($BeSO_4 \cdot 4H_2O$, CAS 7787-56-6, purity 99.99%, Alfa Aesar, Ward Hill, MA, USA). Positive control exposures were prepared by adding appropriate amounts of a solution of beryllium sulfate in ASTM Type I water to SSL soil. Positive controls were included in order to monitor the performance of laboratory culture of the Collembolan *F. candida* and verify consistent sensitivity and response of the test organisms. Glass test containers (42 mm ID; 45 mm deep) were rinsed successively with acetone, tap water, and ASTM Type I water, then allowed to air-dry before the test. In order to prepare replicates of each treatment, 200 g of each air-dried treatment soil, respectively, was hydrated to 88% of the SSL water holding capacity (WHC). Then 23 g of each treatment soil was transferred into a test container and 0.05 g of Baker's Yeast was added to the surface of the soil. Ten 10-to-12-day-old juveniles were placed in each test container, and lightly misted with ASTM type I water. A piece of plastic wrap containing pinholes for air exchange was held in place atop each container with a rubber band. The mass of each container was then recorded, so that soil moisture loss could be monitored, measured, and adjusted weekly during the test. All treatment and control containers were randomly placed in an incubator at 20±0.5°C with RH of

88±5%. During the course of the study, the containers were misted weekly to replace lost water. After 28 days, approximately 15 mL of tap water was added to each test container, and allowed to equilibrate for several minutes. After gentle mixing with a spatula, additional 10 mL water was added to produce a layer of water above the soil. The contents of the test container were then thoroughly mixed again, and subsequently examined under a dissecting microscope (15x) for the presence of juveniles, and adults. Juveniles and adults that floated to the surface of the water were counted. Measurement endpoints were the number of surviving adults, and the number of juveniles produced (the reproduction endpoint), after 28 days of exposure to CFO in SSL soil.

2.4.3.2 Validity of the Test – Collembola

The validity criteria were included in the test as part of the Quality Control procedures. They included the following performance parameters for the negative controls:

1) Adult mortality should not exceed 30% at the end of the test
2) Average number of juveniles per chamber should reach ≥80 instars at the end of the 28-day test
3) Coefficient of variation for reproduction should not exceed 30%.

Test results complied with validity criteria, defined in the ISO 11267 guideline. Adult mortality in negative control was ≤30% and averaged 9±0.3%. Mean production of juveniles in negative control exceeded 80 juveniles, averaging 116 juveniles. The coefficient of variation for the number of juveniles at the end of the definitive test was ≤30%, averaging 11.3%. Production of juveniles in the positive control (50 mg kg^{-1} Be) was decreased by 54% compared to negative controls. Results for positive controls fell within ±2x standard error of the baseline established for the laboratory culture of the Collembolan *F. candida*, as is required for compliance with ISO test guidelines.

2.4.3.3 Data Analysis – Collembola

Student's *t*-Test (two-tailed) with significance level of $p \leq 0.05$ was used to analyze the data from the Limit Test for both the adult survival endpoint, and the juvenile production endpoint. Statistical analyses were done using Microsoft EXCEL software (Microsoft Corporation, 2003).

2.4.4 Limit Microcosm Bioassay

The toxicity of CFO to a natural soil invertebrate community was assessed using a modified version of terrestrial microcosm technique described in Parmelee et al. (1997). The Limit Microcosm Bioassay (LMB); a variant of a multi-concentration definitive test, is used when statistical analyses of pilot test data show no significant effect at all tested treatment concentrations. This LMB consisted of two treatments, negative control (0 mg kg^{-1} CFO) and 1,272 mg kg^{-1} CFO (dry soil); plus high replication (n=8) for increased statistical power. CFO concentrations in soil for all tests were determined from estimated deposition on the basis of the Industrial Code Short Term version 3 (ISCST3) model, which predicted average soil concentrations for distances from source in mg kg^{-1}: 22543 at 10 m, 2056 at 50 m, 336 at 100 m, and 57 at 200 m for a 60-minute dissemination at 3,628 g CFO min^{-1} (STC 2005), assuming an effective depth of incorporation of CFO into SSL soil of 5 cm. The greatest concentrations for single-species and soil community–level toxicity tests are expected to provide a reasonable level of risk assessment, for potentially exposed soil invertebrates beyond 10 and 50 m distance from the source, respectively.

2.4.4.1 Data Analysis – LMB

Student's *t*-Test (two-tailed) with significance level of $p \leq 0.05$ was used in the Limit Test, and to analyze the abundance data for microinvertebrate groups in the microcosm assay, using EXCEL software (Microsoft Corporation, 2003).

3. RESULTS AND DISCUSSION

Investigations showed that uncoated CFO had no significant adverse effects ($p > 0.05$; ANOVA, and Fisher's Least Significant Difference Test) on both adult survival and reproduction by earthworms. Results of ANOVA and LSD tests showed that both number of adults ($p = 1.000$) and number of cocoons ($p = 0.245$) were not significantly reduced in SSL soil amended with concentrations of CFO ≤10,000 mg kg^{-1} compared to control soil. Adult survival was 100% in all treatment levels, including controls. Cocoon production by earthworms in soils amended with 10, 100, 1,000, and 10,000 mg kg^{-1} CFO was 117%, 158%, 108%, and 84%, respectively, compared to cocoon production in control soil.

Results of composite toxicity test with potworm *E. crypticus* showed that CFO material did not significantly affect ($p \geq 0.086$) adult survival at any concentration tested. Juvenile production by *E. crypticus* in the 10,000 mg kg^{-1} treatment was not statistically different ($p=0.796$) from control in the Limit Test. These results confirmed that exposures to CFO $\leq$10,000 mg kg^{-1} in SSL soil did not adversely affect adult survival or juvenile production by *E. crypticus*.

In the invertebrate study using collembolans, test results showed that there was no significant difference ($p=0.379$) between juvenile production in the control group (0 mg kg^{-1} CFO) and juvenile production in the 10,000 mg kg^{-1} CFO concentration. There was no significant effect ($p=0.792$) on adult survival with CFO concentrations $\leq$10,000 mg kg^{-1} in SSL soil.

Soil microinvertebrate community groups, including the indigenous microarthropods Oribatida, Prostigmata, Mesostigmata, and Collembola, plus nematode trophic groups bacterivore, herbivore, fungivore, omnivore, predator, and hatchlings were all unaffected up to the highest concentration tested (1,272mg kg^{-1}). Total abundance of microarthropods was not adversely affected by the soil preparation procedures. Average microarthropod numbers actually increased from 0.44±0.034 ind g^{-1} (n=3) in the baseline samples (assessed immediately after soil collection and prior to soil amendments with CFO) to 0.86±0.089 ind g^{-1} (n=8) in the negative control (Student's *t*-Test p = 0.002) after 14 d. The abundance of nematodes in similarly replicated samples was also greater in negative control (26.1±1.76 ind g^{-1}) after 14 d compared with baseline data (15.5±3.20 ind g^{-1}); the difference was not statistically significant (Student's *t*-Test p = 0.055). These results confirmed that soil preparation procedures and the controlled abiotic environmental conditions in test containers (e.g., soil moisture, temperature, relative humidity, photoperiod, etc.) did not adversely affect the soil microinvertebrate community in SSL soil during assessment of the effects of exposure to CFO material, therefore any significant negative effects from the Limit Microcosm Bioassay may be attributed to CFO.

However, exposure to 1,272 mg kg^{-1} CFO for 14 d did not affect the indigenous microinvertebrate community in SSL soil (Table 2). The total abundance of microarthropods and the abundance of individual microarthropod groups in CFO treatment were not significantly ($p \geq 0.05$) different from negative control. The overall structure of the microarthropod community in SSL soil was not affected by exposure to CFO material based on the number of taxonomic group present in the individual treatments after 14 d (Table 2). The order of relative abundance of microarthropods (from greatest to least) was Oribatida > Prostigmata > Collembola > Mesostigmata in both negative control and CFO treatments (Table 2). Furthermore, miscellaneous taxa of soil arthropods belonging to insect orders Coleoptera, Thysanóptera, and family Formicidae were found in both the CFO and control treatments.

Similar to microarthropods, total numbers of nematodes were unaffected ($p>0.05$) in CFO treatments in SSL soil, compared with negative control. Average abundances of individual trophic groups of nematodes in CFO treatment were not significantly ($p \geq 0.05$) different from negative control. The order of abundance of trophic groups of nematodes (from greatest to least) was bacterivore > fungivore > herbivore > omnivore ≥ hatchling > predator in both negative control and CFO treatment (Table 2). These results show that structure of the nematode community in SSL soil was not affected by exposure to CFO material.

Table 2. Abundance of Microinvertebrate Groups After 14 Days of Exposure to Unamended SSL Soil (Negative Control; no CFO) and to SSL Soil Amended with CFO.

Microinvertebrate Group	Negative control ind g^{-1}*	1272 mg kg^{-1} CFO ind g^{-1}*	p
Microarthropods			
Oribatida	0.45±0.052	0.40±0.047	0.551
Prostigmata	0.31±0.031	0.36±0.045	0.353
Mesostigmata	0.004±0.003	0.007±0.003	0.566
Collembola	0.09±0.021	0.09±0.018	0.920
Other**	0.01±0.006	0.01±0.007	0.767
Total	0.86±0.089	0.87±0.078	0.919
Nematodes			
Bacterivore	11.4±1.26	11.7±1.17	0.839
Fungivore	9.2±1.01	9.8±1.46	0.750
Herbivore	2.6±0.33	1.9±0.22	0.095
Omnivore	1.4±0.17	1.2±0.29	0.608
Predator	0.04±0.04	0.13±0.06	0.278
Hatchling	1.4±0.30	1.1±0.19	0.464
Total	26.1±1.76***	25.9±2.54	0.944

Table notes: Numbers are measurements of abundances of individual groups of soil microinvertebrates, given as means and standard errors (n=8). Probability values (p) were determined using two-tailed Student's *t*-Test (Excel, Microsoft Corporation, 2003).

* Individuals per gram of oven dry soil.

**Miscellaneous groups, including the insect orders Coleoptera (beetles), Thysanóptera (thrips), and family Formicidae (ants).

*** Includes unknown nematode groups, which averaged 0.09 ind g^{-1}.

CONCLUSIONS

Ecotoxicological data established in our investigations indicate acceptable (i.e., no/low) risks to soil invertebrates from exposure to relatively high levels of uncoated CFO. Selected target soil concentrations were based on deposition estimates resulting from a 60-minutes modeled dissemination duration; current M56E1 mission doctrine sets the maximum duration for CFO dissemination at 30-minutes. Therefore modeled deposition data may more accurately depict quantities representative of two consecutive CFO disseminations. These toxicity data are currently included in the Draft MMW P^3I Programmatic Environmental Assessment of CFO. This proactively meets Army goals of matching military mission with wise use of land and natural resources, thus supporting Warfighter readiness through field training and range sustainability. "It is our obligation to ensure that our Soldiers today – and the Soldiers of the future – have the land, water, and air resources they need to train; a healthy environment in which to live; and the support of local communities and the American people" (U.S. Army ASAIE, 2004).

ACKNOWLEDGMENT

This work was supported by the Joint Program Manager for Nuclear Biological Chemical Contamination Avoidance.

REFERENCES

Cataldo, D.A., Driver, C.J., Ligotke, M.W., Landis, W.G., and Norton, M.V.,1992: Environmental and Health Effects Review For Obscurant Fibers/Filaments. CRDEC-CR-126.

ISO, 1998: Soil Quality – Effects of Pollutants on Earthworms (*Eisenia fetida*) – Part 2: Determination of Effects on Reproduction. ISO 11268-2:1998. ISO, Geneva.

ISO, 1999: Soil Quality – Inhibition of Reproduction of Collembola (*Folsomia candida*) by Soil. ISO 11267:1999. ISO, Geneva.

ISO, 2005: Soil Quality – Effects of Pollutants on Enchytraeidae (*Enchytraeus* sp.) – Determinations of effects on reproduction and survival. ISO/CD 16387. ISO, Geneva.

Kuperman, R.G., Checkai, R.T., Simini, M., Phillips, C.T., Kolakowski, J.E. and Kurnas, C.W., 2006: Toxicities of dinitrotoluenes and trinitrobenzene freshly amended or weathered and aged in a sandy loam soil to *Enchytraeus crypticus*, *Environ. Toxicol. Chem*., **25**, 1368-1375.

Kuperman, R.G., Checkai, R.T., Phillips, C.T., Simini, M., Kolakowski, J.E. and Kurnas, C.W., 2005: Weathering and aging of TNT in soil increases toxicity to potworm *Enchytraeus crypticus*, *Environ. Toxicol. Chem*., **24**, 2509-2518.

Kuperman, R.G., Checkai, R.T., Simini, M. and Phillips, C.T., 2004: Manganese toxicity in soil for *Eisenia fetida*, *Enchytraeus crypticus* (Oligochaeta), and *Folsomia candida* (Collembola), *Ecotoxicol. Environ. Saf*., **57**, 48-53.

Kuperman, R.G., Checkai, R.T., Simini, M., Phillips, C.T., Kolakowski, J.E., Kurnas C.W. and Sunahara, G.I., 2003: Survival and reproduction of *Enchytraeus crypticus* (Oligochaeta, Enchytraeidae) in a natural sandy loam soil amended with the nitro-heterocyclic explosives RDX and HMX, *Pedobiologia*, **47**, 651-656.

Kuperman, R., Simini, M., Phillips, C. and Checkai, R., 1999: Comparison of malathion toxicity using enchytraeid reproduction test and earthworm toxicity test in different soil types. *Pedobiologia* **43**, 630-634.

Parmelee, R.W., Phillips, C.T., Checkai, R.T. and Bohlen, P.J., 1997: Determining the effects of pollutants on soil faunal communities and trophic structure using a refined microcosm system, *Environ. Toxicol. Chem.* 16, 1212-1217.

Phillips, C.T., Kuperman, R.G. and Checkai, R.T., 2002: Toxicity of chemical warfare agent HD to Folsomia candida in different soil types. *Eur. J. Soil Biol.* **38**, 281-285.

STC, 2004: Millimeter Wave Pre-Planned Product Improvement ($MMWP^3I$) Air Dispersion Modeling Report #3368. Science and Technology Corporation and Science Applications International Corporation, October 2004 (Contract No.DAAD13-00-/C-0052).

STC, 2005: Addendum to Millimeter Wave Pre-Planned Product Improvement ($MMWP^3I$) Air Dispersion Modeling Report #3368, Annex C. Science and Technology Corporation and Science Applications International Corporation, January 2005 (Contract No.DAAD13-00-/C-0052).

SYSTAT 7, 1997: SPSS, Inc., Chicago, IL, USA.

SYSTAT 10.2, 2004: SYSTAT Software Inc**.** (SSI), Richmond, California, USA.

U.S. Army ASAIE, 2004: The Army Strategy for the Environment: Sustain the Mission – Secure the Future. Office of the Assistant Secretary of the Army For Installations and Environment, 110 Army Pentagon, Room 3E464, Washington, DC 20310-0110

U.S. Department of Agriculture, Natural Resources Conservation Service (USDA/ARS), 1999: Soil Taxonomy (Second Edition), Handbook No. 436. U.S. Government Printing Office, Washington, DC, USA.

USEPA, 1989: Protocols for Short Term Toxicity Screening of Hazardous Waste Sites, Technical Report, EPA/600/3-88/029. Corvallis Environmental Research Laboratory, Corvallis, OR.